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A phase II trial of all-*trans*-retinoic acid in hormone-refractory prostate cancer: a clinical trial with detailed pharmacokinetic analysis

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Abstract Retinoids have been shown to have substantial anticancer activity in a number of preclinical and clinical situations. There are considerable epidemiologic, in vitro and in vivo data which indicate that retinoids may have a role in the prevention and therapy of human prostate cancer. Based on anecdotal evidence of response in one patient with hormone-refractory prostate cancer (HRPC), we conducted a phase II trial in HRPC during which we also examined changes in pharmacokinetics of all-*trans*-retinoic acid (ATRA) which occurred during therapy. Enrolled in the study were 17 patients with HRPC who received 50 mg/m^2 ATRA three times daily orally on days 1–14, repeated every 22 days. The pharmacokinetics of ATRA were assessed with the first dose on day 1, again on day 14 and after a 7-day interruption in ATRA therapy on day 22. Patients were evaluable for response if they completed two 14-day courses of ATRA; among 13 such patients no responses were seen. Four patients were considered unevaluable for response owing to rapid disease progression in three and intercurrent illness in one. Apparent clearance of ATRA changed substantially during therapy: day 1 3779 $\pm$ 4215 ml/min, day 14 7179 $\pm$ 3197 ml/min, day 22 3213 $\pm$ 2357 ml/min. Area under the curve was proportionately diminished on day 14 compared with day 1 and had returned to baseline by day 22. We conclude that ATRA is not active in HRPC. Failure of this agent in HRPC may be related to failure of drug delivery associated with enhanced mechanisms of ATRA clearance which occur within a few days of beginning ATRA treatment.

Key words Prostate cancer · All-*trans*-retinoic acid · Pharmacokinetics · Total body clearance

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Introduction

While androgen deprivation is a modestly effective palliative approach in men with disseminated prostate cancer, progressive disease develops invariably in such men [1, 2]. Extensive drug discovery efforts suggest that new agents such as suramin and novel combinations of agents such as estramustine phosphate (ESM) and vinblastine or ESM and etoposide have activity in men with hormone-refractory prostate cancer (HRPC) [3–5]. Randomized trials to confirm and better quantitate the effectiveness of these agents are being conducted. Nonetheless, new agents are needed for men with HRPC. Additionally, it would be ideal if such agents had a toxicity profile which would allow their use in adjuvant or even preventive situations.

Substantial epidemiologic data suggest that retinoids play a role in the development and progression of prostate cancer[6–8]. Retinoids have important effects on the proliferation of murine prostate tumors and human prostate cells in vitro [9–11]. Furthermore, retinoid analogues inhibit the emergence of prostate cancer in animal systems [12, 13]. We have seen evidence of antitumor activity in one heavily pretreated patient with HRPC who received all-*trans*-retinoic acid (ATRA) [14]. Based on these observations, we evaluated the efficacy of ATRA in HRPC.

The evaluation of the clinical efficacy of ATRA is confounded by the fact that soon after the initiation of ATRA therapy there is marked enhancement of ATRA plasma clearance [15, 16]. This results in substantially lower plasma levels of ATRA after a few days of therapy. In patients with acute promyelocytic leukemia (APL) recurrence of disease has been associated with reduced plasma levels of ATRA [15, 17]. In addition, it appears that patients with epithelial tumors (lung, prostate and head and neck cancers) may have higher baseline clearance of ATRA than patients with APL [18, 19]. These observations are consistent with the hypothesis that ATRA administration is associated with induction of mechanisms responsible for the clearance, and that such a phenomenon may be responsible for reduced drug exposure and failure of ATRA therapy. The mechanisms which account for the change in ATRA clearance with continued administration are unclear. Among the leading possibilities is the induction of increased activity of cytochrome P450 enzymes, a commonly recognized mechanism of inducible drug clearance. ATRA is metabolized by these enzymes in vitro and in animal models [20–23]. We conducted detailed serial pharmacokinetic studies and clinical assessment of the activity of certain P450 isozymes in patients treated with ATRA in order to gain greater understanding of the development of enhanced clearance of ATRA in patients with prostate cancer and the role this might play in the clinical activity of ATRA.

Materials and methods

Patient eligibility

Patients entered on this trial were required to meet the following eligibility criteria: histologic diagnosis of adenocarcinoma of the prostate and manifestations of progressive disease despite initial endocrine therapy (bilateral orchiectomy, estrogens or luteinizing hormone-releasing hormone [LHRH] therapy). Adequate renal (serum creatinine less than 2.0 mg/dl), bone marrow (granulocyte count greater than 1500/mm^3, platelet count greater than 100 000/mm^3), and hepatic function (bilirubin less than 2.0 mg/dl, serum glutamic oxaloacetic transaminase [SGOT] less than 4 × normal) were required. A performance status (PS) of 2 or better using the Eastern Cooperative Oncology Group (ECOG) criteria was required. This study was approved by the University of Pittsburgh Biomedical Institutional Review Board and written informed consent was obtained. Patients were required to have an estimated survival of at least 8 weeks. Men who had not undergone orchiectomy, were required to have a serum testosterone concentration within the castrate range (< 20 ng/ml) prior to the study. An evaluable disease parameter was required. These parameters consisted of tumor masses measurable or evaluable by standard ECOG criteria [24] or an increased serum prostate-specific antigen (PSA). Patients were considered ineligible for this trial if there was a history of any other malignancy within the preceding 24 months with the exception of nonmelanoma skin cancer or noninvasive superficial bladder cancer; radiation therapy must have been completed at least 14 days prior to study entry. Prior cytotoxic therapy was an exclusion criterion for this study. Prior therapy with ESM (Estracyt) was allowed. Patients receiving flutamide were required to have discontinued flutamide 4 or more weeks prior to study entry and to have demonstrated continued disease progression despite flutamide withdrawal. Men on estrogens or LHRH analogues for testicular androgen suppression continued these agents during study.

Treatment with ATRA

ATRA was supplied by the Investigational Drug Branch, National Cancer Institute in opaque gelatin capsules containing 10 mg ATRA. ATRA was administered orally to all patients at a dose of 50 mg/m^2 three times daily (150 mg/m^2 total daily dose) for 14 days. Therapy was then interrupted for 7 days and was reinitiated on day 22. This schedule (14 days on/7 days off) was continued until progression or limiting toxicity occurred. Doses of ATRA were reduced for the occurrence of grade 2 or greater toxicity (NCI Common Toxicity Criteria).

Response Assessment

Patients were assessed for response in measurable or evaluable disease according to standard ECOG response criteria [24]. Response in PSA was judged as follows: complete response, normalization of PSA persisting for 6 weeks or more; partial response, $\geq 50\%$ decrease lasting for 6 weeks or more; and progression, $\geq 50\%$ increase documented on two determinations at least 2 weeks apart. PSA response was defined as having begun when a single PSA value constituting response (normalization or $\geq 50\%$ decrease) was obtained providing that degree of PSA change was maintained for ≥ 6 weeks from the date the initial decrease was seen.

Patient evaluation

After the initial 21 days of protocol therapy patients were seen and evaluated every 21 days. Bone scans were repeated every 12 weeks; PSA was determined every 21 days. Complete blood counts, liver and renal function tests were performed every 21 days.

Pharmacokinetic analysis

To serially assess the effects of the duration of therapy on ATRA disposition, blood samples for pharmacokinetic analysis were drawn from patients after the first dose of ATRA (baseline), after 14 days of therapy (treatment), and on day 21, after a 1-week break from therapy (washout). ATRA was administered orally along with 500 ml of a standardized liquid diet consisting of 30% fat, 5% protein, and 65% carbohydrate. Blood samples were drawn through a venous catheter into heparinized Vacutainer tubes covered with aluminum foil for light protection. Collection times were immediately before and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 11, and 12 h after the ATRA dose. The samples were centrifuged at 2000*g* for

10 min at 4 °C and the plasma aliquoted into yellow polypropylene tubes and stored at −75 °C until analyzed.

The plasma concentrations of ATRA, 4-oxo ATRA and retinol were determined by a modification of the method described by Bugge et al. [25]. Standard ATRA, etretinate, and 4-oxo ATRA were provided by Hoffman La-Roche. Retinol was purchased from Eastman Laboratory Chemicals (Rochester, N.Y.).

Retention times for 4-oxo ATRA, etretinate, ATRA, and retinol were 11.9, 20.0, 25.6, and 26.5 min, respectively. 4-Hydroxy-ATRA and 13-*cis*-RA had retention times of 10.6 and 24.1 min respectively. Detection was by UV absorption at a wavelength of 365 nm. ATRA 4-oxo ATRA, and retinol were quantitated by comparing peak height ratios (drug/internal standard) for unknowns with those obtained from standards prepared in an artificial plasma consisting of 5% bovine serum albumin in 0.9% saline. Standard curves were linear ($r^2 \geq 0.998$) over all concentration ranges studied (ATRA 10–1000 ng/ml, 4-oxo ATRA 10–400 ng/ml, and retinol 40–100 ng/ml).

Pharmacokinetic analysis focussed on parameters which could be assumed to be reasonably unaffected by the dramatic variability in the timing of absorption of ATRA. This variability was manifest by substantial bimodality of several concentration-time curves and the irregular shape of several others. These parameters included AUC, and the elimination rate constant. To take into account the variability in absorption, the elimination rate constant was estimated from the maximum negative slope.

Statistical analysis

The primary goal of this study was to evaluate the clinical activity of ATRA in patients with prostate cancer. An optimal, two-stage design was used to test the hypothesis: H_0 $p \leq 0.15$ versus H_a $p \geq 0.35$, where p is the proportion of patients who responded to treatment after receiving at least 14 days of continuous therapy. In the first stage of the study, 15 evaluable patients were to be entered. Patients who were not evaluable for response were replaced. If at least 3 of the first 15 patients evaluable for response showed a response, then 22 additional patients were to be entered, (total 37 patients). If fewer than 3 of the first 15 patients responded, the trial was to be terminated.

Assessment of pharmacokinetic parameters, immune parameters, and metabolic enzyme parameters was performed by Spearman correlation analysis, Mack-Skillings tests, Friedman nonparametric analyses of variance, signed-rank tests, and multiple regression. Parameters were transformed to reduce non-normality and logarithmic transformation was applied to AUC, and NK lytic units to reduce heteroscedasticity.

Immunologic studies

Retinoids have been reported to have effects on immune parameters. Consequently, we assessed the following immunologic parameters before therapy, on day 2, day 15 and day 22: total lymphocyte, monocyte, and granulocyte count, and percentage and absolute number of cells positive for CD3, CD8, TAC, CD4, Leu13, CD16 and CD56, Leu 20, Leu12 and Leu20, p75, DR and Leu23. These assays were done by flow cytometry in the Immune Monitoring Laboratory (IML) of the University of Pittsburgh Cancer Institute.

Results

Clinical data

A group of 17 men were enrolled on this trial; all were Caucasian. Their mean age was 63 years (range 42–84 years). Ten had a PS of zero and seven had a PS of 1. All had prostate cancer with the following distribution of metastases: bone only (11), bone and regional nodal metastases (2), bone and skin (1), hepatic (1) or only regional nodal metastases (2).

Serum PSA was evaluated in 15 of 17 patients at entry (median 68.6 ng/dl range 18.4–542.7 ng/dl). In two men PSA was <0.1 ng/dl, each of whom had a well-documented prior diagnosis of prostate cancer, had received definitive local therapy (irradiation 1, radical prostatectomy 1) but had experienced recurrence with skin and bone metastases (1) or regional nodal metastases. Biopsy of recurrent masses in each man disclosed adenocarcinoma consistent with the original prostate tumor. Careful evaluation disclosed no evidence of other primary cancers in either man. All men had undergone androgen deprivation therapy with either orchiectomy or LHRH analogues, and 12 men had received flutamide. Patients were considered evaluable for response if they completed two 14-day cycles of ATRA; all patients were evaluable for toxicity. Four patients were considered unevaluable for response, three because of progressive disease requiring marked increase in analgesics and irradiation therapy for painful bone lesions 16, 14 and 15 days after beginning ATRA. One man had a stroke on day 12 of therapy and was unable to continue treatment. This stroke was not felt to represent a toxicity of ATRA.

Among the remaining 13 patients, none showed any evidence of response. Progression, determined by a greater than 50% increase in PSA and other evidence of disease progression (increase in pain, new lesion on bone scan or increase in soft tissue disease), was seen in 11 patients. In two patients with nodal or skin metastases and normal PSA, clear progression in soft tissue disease was documented. Median time to progression was 62 days (range 36–167 days). Median survival of all patients entered was 354 days.

Toxicity

Toxicity of ATRA was mild and manageable. Most patients noted xeroderma, cheilosis and mild conjunctivitis. Two men developed painful balanitis controlled with emollients. Six men noted headache; it was mild in two and moderate in four. One man required a 50% ATRA dose reduction because of intolerable headache occurring on day 1 of therapy. No other important toxicities were noted.

ATRA pharmacokinetics

Following oral administration of the first dose of ATRA, plasma concentrations rapidly rose to a peak by 2 h, remained at a plateau, and then declined rapidly (Fig. 1). If complete gastrointestinal absorption was

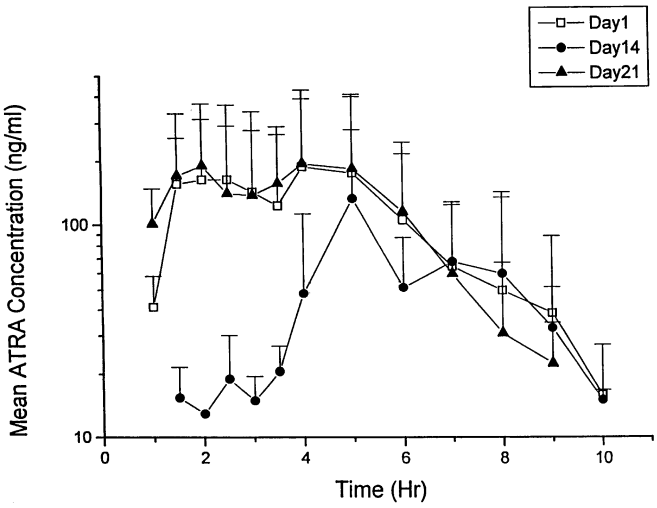

Fig. 1 Plasma concentration-time curves for 17 subjects treated orally with ATRA (50 mg/m^2) on days 1, 14, and 21. Day 1 data were obtained following the first dose, day 14 after 2 weeks of continuous therapy, and day 21 after a 1 week break from therapy (mean ± SD)

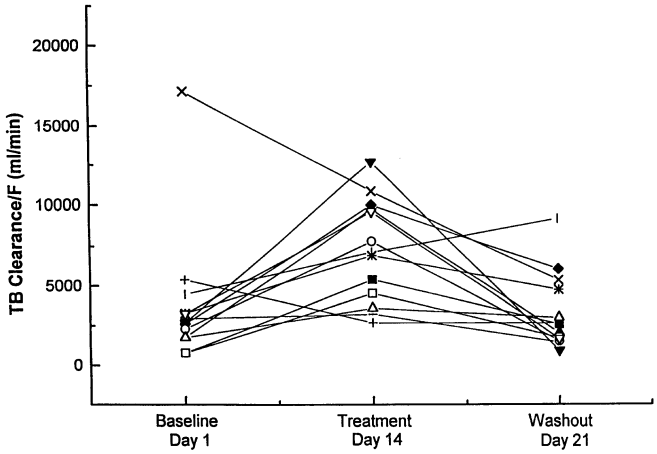

Fig. 2 Plasma clearance values for 17 individual subjects treated orally with ATRA (50 mg/m^2) on days 1, 14, and 21

assumed, the apparent oral clearance was high with wide intersubject variation (Fig. 2, Table 1). This suggests a high presystemic elimination. However, we are unaware of any data addressing the issue of completeness of absorption. Therefore, the apparent high presystemic elimination may include an indeterminate contribution from incomplete absorption. Such incomplete absorption could consist of enteric metabolism as well as "failed absorption".

Following 14 days of ATRA therapy, there was a substantial change in the appearance of the plasma concentration-time profile (Fig. 1). Plasma concentrations were lower, peak concentrations were detected later and the peak concentration was lower on day 14 compared with day 1; however, the terminal half-life

Table 1 Pharmacokinetic parameter estimates for ATRA in 17 prostate cancer patients treated with 50 mg/m^2 three times daily for 14 days (T_{first} time at which ATRA initially detectable after oral administration). Values are presented as the means ± SD with ranges in parentheses

	Day 1 (baseline)	Day 14 (treatment)	Day 21 (washout)
C_{max} (ng/ml)	295 ± 214 (29–742)	146 ± 116†* (31–469)	292 ± 188 (39–771)
T_{max} (h)	3.88 ± 1.93 (2.0–8.0)	6.07 ± 1.75†* (2.0–9.0)	3.96 ± 1.19 (2.0–6.0)
$T_{1/2}$ (h)	1.15 ± 0.61 (0.73–2.86)	0.83 ± 0.17 (0.59–1.00)	0.76 ± 0.18 (0.55–1.24)
T_{first} (h)	1.88 ± 0.84 (1.0–3.5)	4.23 ± 1.37†* (1.0–6.0)	2.38 ± 1.32 (1.0–5.0)
AUC (ng × h/ml)	725 ± 515 (107–1901)	276 ± 183†* (88–752)	687 ± 422 (166–1730)
Clearance (ml/min)	3779 ± 4215 (788–17133)	7179 ± 3197†* (2438–12605)	3213 ± 2357 (867–9037)

†$p \leq 0.05$ day 1 vs day 14, *$p \leq 0.05$ day 21 vs day 14

was unchanged. Thus, the overall AUC was decreased. These changes are characteristic of what would be anticipated with an increase in apparent oral clearance (Fig. 2) and increase in presystemic drug elimination.

After 14 days, ATRA therapy was discontinued for 7 days, and then reinstituted for the next cycle of therapy. The plasma AUC with the first dose of reinstituted therapy showed a return toward concentrations and the concentration-time profile seen on day 1 (Figs. 1, 2, Table 1).

Several metabolic byproducts of ATRA were observed in the chromatograms generated from the analysis of plasma samples from ATRA-treated subjects. Figure 3 shows representative chromatograms from a single subject. Figure 3A shows the chromatogram obtained from the analysis of a plasma sample prior to initiating ATRA therapy. Identifiable peaks include retinol (peak 8) and the added internal standard, etretin (peak 6). After the initial dose of ATRA on day 1 several additional peaks can be seen in the plasma sample collected at the C_{max} time-point for this subject. In addition to the peaks described previously, additional identifiable analytes were observed. Peak 7 corresponds to synthetic ATRA standard while peak 5 corresponds to 4-oxo ATRA. Peak 4 is presumed to be 4-hydroxy ATRA based on the retention time of synthetic standard. Unknown metabolites of ATRA are shown as peak areas 1 and 2 as well as peak 3. In previous animal or in vitro studies with ATRA, these metabolites are frequently referred to as the "polar fraction". Almost no in vivo conversion of ATRA to 13-*cis*-RA occurred with any of the subjects in this trial. Figure 3C demonstrates the return to baseline conditions for this subject several hours after the peak plasma concentration (Fig. 3B) was observed. The

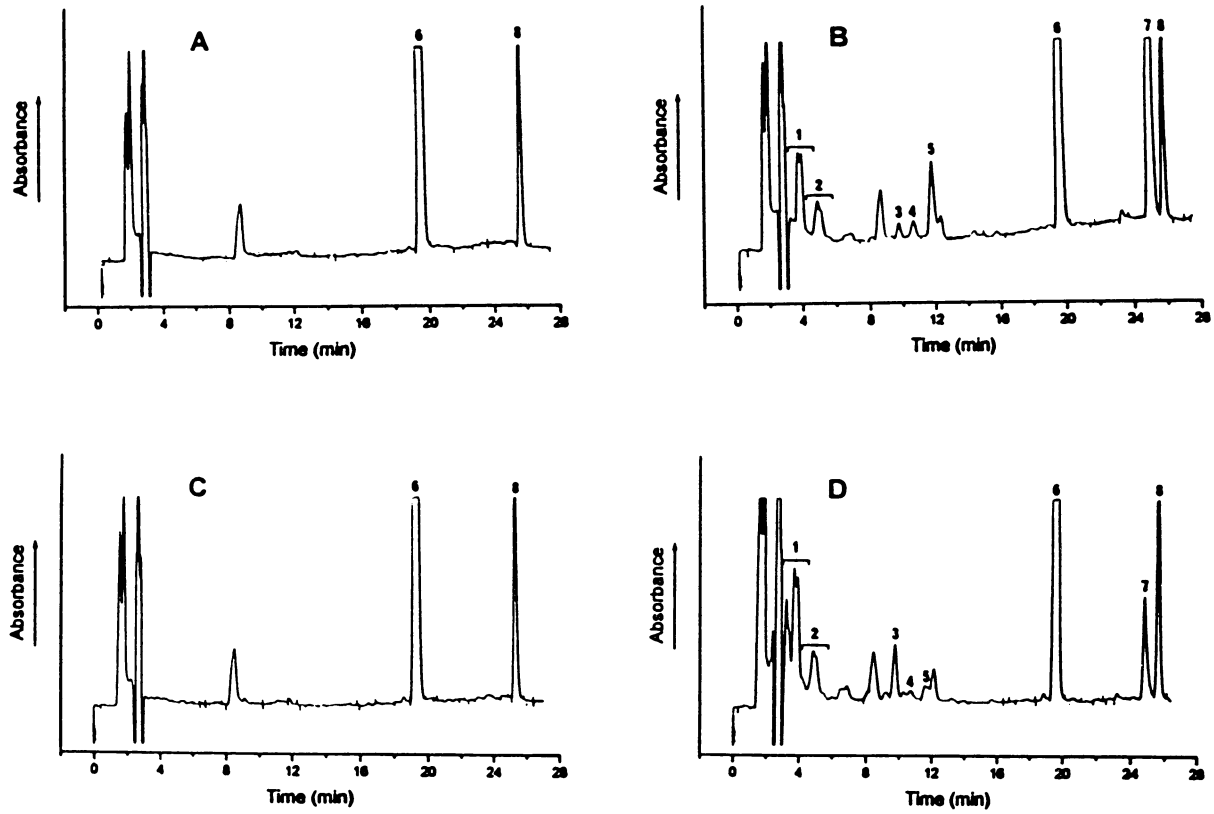

Fig. 3A–D HPLC profile of ATRA, retinol, 4-oxo ATRA, and other metabolites isolated from the plasma of a patient treated with ATRA. **A** Predose chromatogram; **B** day 1, C_{max} time-point after a 50 mg/m^2 dose; **C** day 1, last sample time-point; **D** day 14, C_{max} time-point after a 50 mg/kg dose (*peaks* 1, 2, *and* 3 unknown polar metabolites, *peak* 4-hydroxy ATRA, *peak* 5 4-oxo ATRA, *peak* 6 internal standard, *peak* 7 ATRA, *peak* 8 retinol)

chromatogram shown in Fig. 3D was obtained from the analysis of the plasma sample at the C_{max} of this subject on day 14 after chronic administration of ATRA. As is clearly seen, the ATRA peak was substantially smaller than that observed on day 1. The 4-oxo ATRA peak was also depressed. However, peak areas 1 and 2 as well as peak 3, representing possible ATRA metabolites, were greater than those observed on day 1 even though the concentration of the parent compound ATRA was greatly decreased.

Measurement of 4-oxo ATRA in all plasma samples indicated much lower concentrations and AUC than of parent drug (data not shown). The ratio of metabolite to parent drug, however, does not provide a measure of the relative importance of this route of metabolism, as concentrations are a function of production rate, as well as distribution and elimination of metabolite. However, the ratio does provide an indirect estimate of whether the relative proportion metabolized via this route remains constant. Comparison between day 1 and day 14 indicated that the AUC of both parent drug and metabolite changed, with the ratio remaining unchanged, suggesting that this route of metabolism was changed in proportion to the overall induction of metabolism.

Retinol

Retinol concentrations for each treatment phase were calculated as the mean of the 0- and 0.5-h blood samples; these were time periods prior to the apparent absorption of ATRA. On days 1, 14, and 21 retinol concentrations varied greatly among the individuals although there was no significant difference in the mean retinol concentrations between days. The mean retinol concentrations on days 1, 14, and 21 were 705, 732, and 731 ng/ml, respectively.

Hematologic and immune parameters

ATRA administration was associated with a clear decrease in white blood cells ($P = 0.003$), on day 2, as well as on day 15 and day 22; the median decrease was 12–20%. Granulocytes were decreased both in absolute number and in terms of percentage. Absolute lymphocyte count increased on day 2, then returned to

baseline. Monocyte counts were not affected by treatment. In studies of lymphocyte markers, it was found that T-cell markers (CD3, CD4, CD8) tended to increase after treatment (day 2). B cell markers and activation markers (DR, TAC, p75) were not affected. Exploratory analysis suggested a relationship between ATRA maximal plasma concentration on day 1 vs day 14 and a decrease in granulocyte count on day 1 vs day 14. No other measures of ATRA exposure appeared to influence hematologic parameters.

Discussion

Retinoids are a class of compounds consisting of vitamin A and numerous analogues. They have very important effects on the growth and development of mammalian epithelial tissues [26–28]. Retinoids have shown clear activity as anticancer agents. Topical application of ATRA is effective in the therapy of epithelial atypia in the uterine cervix and oral 13-*cis*-RA as a single agent appears to be effective in the prevention of second primary head and neck cancers in individuals who have had one primary [29, 30]. In combination with interferon-α, 13-*cis*-RA is active in causing regression of advanced squamous cell carcinomas of the uterine cervix and skin [31]. ATRA is a very effective agent in remission induction in APL [32, 33].

Considerable evidence indicates that retinoids may be important in prostate cancer. Reichman et al. studied serum retinol content among 2440 men over the age of 50 years [6]. At 10-year follow-up the risk of developing prostate cancer was 2.4 times higher among men whose plasma retinol content was in the lowest quartile of the population compared with those in the highest quartile ($P < 0.012$). This study is important in that it is the only *prospective* trial in which retinoids have been measured in a sufficient number of normal men to identify a substantial number of subsequent cases of prostate cancer.

Many workers have reported important effects of retinoids on growth of normal and neoplastic prostatic epithelium [9–11]. In tissue derived from patients with benign prostatic hypertrophy, retinol acetate (3×10^{-9} *M*) inhibits epithelial proliferation which is stimulated by epidermal growth factor [EGF]. Of note, 3×10^{-8}–3×10^{-7} *M* retinol acetate actually enhanced EGF-mediated proliferation. At pharmacologic concentrations, retinoids inhibit the proliferation and clonogenicity of the human prostate cancer cell lines, PC-3 and LNCaP [10, 11].

There are also data indicating important effects of retinoids in in vivo prostate systems. Lobund-Wistar rats develop prostate cancer at an unusually high rate (26%) and treatment of 3-month-old rats with methylnitrosourea (MNU) followed by continuous supplemental testosterone results in the development of cancer arising in the prostate and seminal vesicles in 70–90% of rats. MNU-treated rats treated with the retinoid, 4-hydroxyphenylretinamide (4-HPR) show marked reduction of tumor development (21%) [12]. 4-HPR shows similar effects in suppressing prostate carcinogenesis in Thompson's reconstitution model [13]. Pienta et al. have recently reported that 4-HPR is effective in the therapy of established tumors in animals with Dunning adenocarcinoma of the prostate [34].

Despite these encouraging preclinical results, there is little evidence of a beneficial effect of ATRA in humans. ATRA was ineffective in patients with HRPC in the present study. Kelly et al. have also reported a negative trial of ATRA in HRPC [35]. The finding that clearance of ATRA increases rapidly after initiation of therapy suggests that failure of ATRA in prostate cancer may be related to inadequate drug exposure as a result of inadequate drug delivery.

Studies by investigators at the Memorial Sloan Kettering Cancer Institute as well as other groups have confirmed the induction of enhanced ATRA clearance in patients with APL as well as other tumors [15, 17]. Limited but provocative data from these workers suggest that ATRA clearance may be more rapid constitutively in patients with solid tumors such as lung cancer [18]. Our data in prostate cancer patients also indicate that ATRA clearance is rapid prior to any exposure. Adamson et al. have reported that enhanced clearance mechanisms of ATRA are activated within 3–5 days of beginning therapy [36].

A prime candidate mechanism of enhanced ATRA clearance is through induction of cytochrome P450 enzymes. Retinoids, including ATRA, are metabolized by P450 isozymes in vitro [21–23]. P450 inhibitors such as ketoconazole and liarazole acutely enhance ATRA plasma levels in patients who have been receiving ATRA for 28 days [18, 19]. However, this effect of ketaconazole does not persist when ketoconazole is administered at a dose of 200 mg three times daily for 14 days [37]. Muindi et al. have presented data to suggest that lipoxygenese activity is enhanced in conjunction with ATRA administration [38]. It is possible that lipoxygenese or cyclooxygenase enzyme induction may contribute to enhanced ATRA clearance.

Mechanisms other than metabolism may also contribute to enhanced ATRA clearance. Cellular RA binding proteins (CRABP) are increased within a few days of ATRA administration. CRABP binds and sequesters ATRA. CRABP induction may contribute to enhanced plasma clearance of ATRA and hence ATRA "resistance" [39].

In summary, there is strong preclinical information to suggest that ATRA or other retinoids could have therapeutic benefit in HRPC. The lack of response in the present study should not be used to infer that this strategy has no relevance to humans. There is an urgent need to better understand the complexities of the mechanisms involved, of retinoid action and to explore the

use of other agents, as well exploring approaches to enhance ATRA delivery. Among the available options for enhancing ATRA delivery are encapsulation in liposomes or concomitant administration of inhibitors of ATRA metabolism.

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